Carbohydrate Polymers 165 (2017) 39–50

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Salicyl-imine-chitosan hydrogels: Supramolecular architecturing as a crosslinking method toward multifunctional hydrogels

Manuela-Maria Iftime, Simona Morariu, Luminita Marin*

"Petru Poni" Institute of Macromolecular Chemistry of Romanian Academy – 41A, Aleea Gr. Ghica Voda, Iasi, Romania

ARTICLE INFO

Article history:
Received 13 December 2016
Received in revised form 23 January 2017
Accepted 8 February 2017
Available online 10 February 2017

Keywords:
Chitosan
Dynamic covalent chemistry
Superporous
Luminescence
Thixotropy
Self-healing

ABSTRACT

Hydrogels based on chitosan and salicyladehyde were obtained by dynamic covalent chemistry. The unusual chitosan gelling in the presence of the monoaldehyde has been deciphered following and correlating data of NMR, FTIR, single crystal and wide angle XRD, POM and optical measurements. Of significant importance in understanding the crosslinking features was the synthesis of a model compound and the successfully growth as single crystal allowing the study of its supramolecular peculiarities. The hydrogels exhibited in SEM a porous or fibrous morphology, in good correlation with the crosslinking degree. They swelled very fast, similar to the superporous hydrogels of third generation and exhibited self-healing properties. Rheological investigation demonstrated good mechanical properties, thermosensitivity and thixotropy. The paper revealed a hydrogel with suitable properties for use in bio-medical applications, and moreover, revealed a new concept of obtaining chitosan hydrogels using monoaldehydes – which are widespread in nature, cheap and beneficial to the human body.

1. Introduction

Hydrogels are a class of materials applied in many fields of human life, especially in those bio-related: substrates for biomedical engineering and pharmacology, environment protection, agriculture, food industry, hygiene and so on (Ahmed, 2015; Caló & Khutoryanskiy, 2015; Shen, Shamshina, Berton, Gurau, & Rogers, 2016). Advances in hydrogel domain intersected the modern domain of supramolecular chemistry, moving from static to dynamic complexity in hydrogel design. Dynamic (hydro)gels were prepared based on reversible reactions, of physical or chemical nature: acylhydrazone bonds (Deng, Tang, Li, Jiang, & Chen, 2010), Schiff bases (Zhang, Tao, Li, & Wei, 2011), reversible ring-opening of 1,2 dithiolanes (Barcan, Zhang, & Waymouth, 2015), boronic ester transesterification (Brooks & Sumerlin, 2016), thiol–disulphide interconversion (Casuso et al., 2015), metalophilic attractions (Casuso et al., 2014), metal ligand exchange (Sreenivasachary & Lehn, 2005) or telechelic dendritic macromolecules with multiple adhesive termini (Wang et al., 2010). The use of reversible connections for crosslinking led to a new generation of smart materials/hydrogels able to respond to environmental stimuli or artificial triggers. They usually exhibit self-healing behaviour and have the capability to recover quickly their mechanical properties after removing the stress.

Among various reversible covalent connections used for obtaining dynamic materials, the condensation of amino groups with carbonyl functionalities to yield imines also known as Schiff bases or azomethines is considered the most powerful strategy used in dynamic covalent chemistry to generate structures of high complexity, dynamic nanoarchitectures and materials with modulable properties (Liu & Lim, 2013; Roy, Bruchmannb, & Lehn, 2015) or in dynamic combinatorial chemistry to trigger the easy selection of components as response to external physical stimuli or chemical effectors (Clima, Peptanariu, Pinteala, Salic & Barboiu, 2015; Sreenivasachary & Lehn, 2005; Turin-Moleavin et al., 2015; Zhang & Barboiu, 2016). The advantages of reversible imine linkage consist mainly in its fast exchange tuned by the reagent reactivity, the presence of water, the pH or the temperature. Moreover, the equilibrium of the imine exchange is an active intermediate/target in many biological processes and pharmaceutical chemistry as enzyme catalysis (Fisher & Viswanathan, 1984), signaling through G-protein-coupled receptors (Perez & Karnik, 2005), transamination, obtaining of biomarkers or reaction of sugars with biological relevant amines (Qin, Long, Panunzio, & Biondi, 2013). From these reasons, the reversible imine connection is of great interest in chemistry, biology and materials science and was implemented for the search of biologically active substances (Marin et al., 2016; Nasr et al., 2009; Clima, Peptanariu, Pinteala, Salic, & Barboiu, 2015;

* Corresponding author.
E-mail addresses: lmarin2011973@yahoo.com, lmarin@icmpp.ro (L. Marin).

http://dx.doi.org/10.1016/j.carbpol.2017.02.027
0144-8617/

Ramstrom, Lohmann, Bunyapaiboonsri & Lehn, 2004; Roy et al., 2015; Turin-Moleavin et al., 2015; Zhang & Barboiu, 2016).

In this context, preparation of dynamic chitosan hydrogels based on reversible imine connections appears as a promising and challenging design. Chitosan is a naturally derived, biocompatible polymer which already proved high applicability in biochemistry and bioengineering (Bhattarai, Gunn, & Zhang, 2010; Delmar & Bianco-Peled, 2016; Giri et al., 2012; Nawrotek, Tylman, Rudnicka, Balcerzak & Kamiński, 2016). Some previous preliminary studies of our group noticed the possibility of chitosan gelling with some monoaldehydes (Marin, Simionescu, & Barboiu 2012; Marin et al., 2015), by a dynamic process of physico-chemical crosslinking (Ailincai et al., 2016; Marin et al., 2014). The topic is of high interest taking into consideration that monoaldehydes exist in a large variety, many of them being natural products with therapeutic properties, compared to the dialdehydes which are generally used for chitosan crosslinking which were proved to have a toxicity degree (Beauchamp, Clair, Fennell, Clarke, & Morgan, 1992; Berger et al., 2004; Mikhailov et al., 2016). Thus, hydrogels obtained from natural products as chitosan and natural aldehydes should have better premises for safer bio-related applications. Herein, we explored the obtaining of hydrogels based on the natural products, chitosan and salicylaldehyde, in order to provide a hydrogel appropriate for bio-applications, and to pave a way of chitosan crosslinking by a new friendly method. Salicylaldehyde has been chosen as possible chitosan crosslinker due to its intrinsic properties. Natural occurring in buckwheat seeds, exudate from castor sacs or larval defence secretions of several beetle species, salicylaldehyde was approved by the U.S. Food and Drug Administration (FDA) and Flavour and Extract Manufacturers Association (FEMA) (Adams et al., 2005; Salicylaldehyde, 1979) for food use. It was demonstrated that it has antifungal, anti-mycotoxigenic and chemosensitizing properties and is evaluated in the chemotherapy of invasive fungal diseases and in agriculture as inhibitor of fungal growth and mycotoxin production (Kim, Campbell, Mahoney, Chan, & Molyneux, 2011). Moreover, studies on its imine derivatives proved antimicrobial and antifungal properties (da Silva et al., 2011; de Araújo, Barbosa, Dockal, & Cavalheiro, 2017; Zaltariov et al., 2015) and even anticancer features (Garrick et al., 1991).

Two aspects must be highlighted here: (i) chitosan gelling with monoaldehydes is a new topic in the chitosan chemistry which development was started by us; (ii) even if a few papers reported the obtaining and the properties of imine derivatives based on chitosan and salicylaldehyde [e.g. de Araújo et al., 2017; Menaka & Subhashini, 2016; Deng, Fei, & Feng, 2011; dos Santos, Dockala, & Cavalheiro, 2005), there are no studies in literature dedicated to the salicyl-imine-chitosan hydrogels, neither on their formation or their properties. The study reported here reveals in fact a novel hydrogel with good mechanical properties, thermosensitivity, thixotropy, self-healing, fast swelling, and luminescence keeping the promise for bio-applications.

2. Experimental section

2.1. Materials

Low molecular weight chitosan (263 kDa) with a degree of deacetylation (DA) of 83%, salicylaldehyde 98%, D-glucosamine hydrochloride 99%, ethanol, glacial acetic acid and phosphate buffer solution of pH 7.4 were purchased from Sigma–Aldrich Co. (USA) and were used as received. The number of the free amino groups of chitosan was calculated on the basis of DA. Acetate buffer solution of pH 4.2 was prepared as described by Lambert and Muir (Lambert & Muir, 1973). Bidistilled water was obtained in the laboratory.

2.2. Synthesis of the model compound: 2((o-hydroxybenzylidene)amino)-5-(hydroxymethyl) tetrahydro-1H-pyran-1,3,4-triol (MC)

D-glucosamine hydrochloride, (0.3 g, 1.39 mmol) was suspended in methanol (4.41 mL) with 1 equiv. of finely grinded solid NaOH (0.058 g, 1.39 mmol). After 5 min, the resulted NaCl was filtered off. Salicylaldehyde (0.043 mL, 1.39 mmol) was slowly added to the filtrate, and the reaction mixture was kept at ~35 °C, up to a yellow solid precipitated, after 5 min. The mixture was cooled in an ice bath; the solid was filtered off, washed with ice-cold methanol and dried under vacuum for 2 days (Costa Pessoa, Tomaz, & Henriques, 2003; Nguyen, Nguyen, Ho, & Ngo, 2011). The yellow powder was recrystallized from ethanol to give yellow needles single crystals with a yield of ~10%.

Single crystal of **MC** was mounted in inert oil and transferred to the cold gas stream of the diffractometer. Structure determination of crystal data: $C_{13}H_{17}NO_5$: $M_r = 267\ g\ mol^{-1}$; space group I2; cell lengths: a = 13.1519(8) Å b = 6.0602(2) Å, c = 17.1108(11) Å; cell angles: $\alpha = 90^\circ$, $\beta = 105.219(6)^\circ$, $\gamma = 90^\circ$; cell volume V = 1315.96 Å^3. The right structure has been confirmed by FTIR, ^{1}H NMR and ^{13}C NMR spectra, too (Fig. 1s, 2s).

2.3. Preparation of the hydrogels and xerogels

The synthesis of the hydrogels was carried out by acid condensation reaction of the chitosan with salicylaldeyde. Briefly, a 1% solution ($g\ mL^{-1}$) of salicylaldehyde in ethanol (Table 1) was added drop wise to a 2% solution ($g\ mL^{-1}$) of chitosan (0.3 g, 1.473 mmol glucosamine repeating units) in acidic water (0.7% acetic acid solution: 105 μL of acetic acid in 15 mL of water), under vigorous magnetic stirring (750 rpm), at 50 °C. The molar ratio between the NH_2 and CHO functional groups has been varied, keeping constant the amount of chitosan and changing the amount of aldehyde to achieve hydrogels with different crosslinking densities (Table 1). The hydrogels appeared as transparent yellowish semisolid materials with smooth texture, without air bubbles or other macroscopic particles, as also Cavalheiro noticed (de Araújo et al., 2017). The visual formation of hydrogels was observed after 8 min in the case of 1/1 molar ratio of the NH_2/CHO functional groups (**S1**), after 3 h in the case of 1.5/1 (**S1.5**) and of 2/1 (**S2**) respectively, and after 1 week in the case of 2.5/1 (**S2.5**). For a lower amount of aldehyde (NH_2/CHO = 3/1 (**S3**); 3.5/1(**S3.5**)) the reaction medium transformed into a viscous liquid which still flew even after 2 weeks. All hydrogels were kept uncovered for two days up to the initial volume of chitosan solution was reached. According to the NMR analysis which indicated the increase of the conversion of the amine groups into imine linkages during 16 days, the hydrogels were further kept covered another two weeks for gelling maturation. The corresponding xerogels of the obtained hydrogels were prepared by lyophilization. A 2% chitosan solution in 0.7% acetic acid has been also lyophilized, to be used as reference (**S0**). The xerogels weight was similar with that of the initial reagents, indicating no mass loss during lyophilization.

2.4. Methods

In order to analyse the structure and morphology of the hydrogels, the corresponding xerogels were obtained by freezing in liquid nitrogen and further submitted to lyophilization using a LABCONCO FreeZone Freeze Dry System equipment, for 24 h, at −54 °C and 1.510 mbar.

FTIR spectra of the xerogels were registered using a FT-IR Bruker Vertex 70 Spectrofotometer, by ATR technique and processed using Origin8 software.